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Effect of Aqueous Seed Extract of *Aframomum Melegueta* on Insulin Concentration in Alloxan-induced Diabetes in Male Wistar Rat

Mmuogbo, J. C; Maduka, S. O.; Okonkwo, O. C.

Department of Human Physiology, Faculty of Basic Medical Sciences,
College of Health Sciences, Nnamdi Azikiwe University, Awka, Nigeria

ABSTRACT

Aframomum Melegueta (Grain of paradise) is a perennial herbal plant that is cultivated for its valuable medicinal and pharmacological effects. The study aims to determine the effect of aqueous seeds extract of *Aframomum Melegueta* on insulin concentration in alloxan-induced diabetes in male wistar rats. Twenty-five (25) male wistar rats weighing 150g -170g were used in this study. The animals were acclimatized for a period of two (2) weeks, after which they were randomly divided into five (5) groups of five rats each. Group A served as negative control and received feed and water ad libitum, group B served as positive control and received alloxan monohydrate, group C received 120mg of alloxan monohydrate and was treated with 150mg of aqueous seed extract of *Aframomum Melegueta,* Group D received 120mg of alloxan monohydrate and was treated with 300mg of aqueous seed extract of *Aframomum Melegueta* and Group E received 120mg of alloxan monohydrate and was treated with 600mg of aqueous seed extract of *Aframomum Melegueta*. The Administration of the extract last for a period of 21 days and The administration was between the period of 6 am to 8 am every day. Data for blood glucose and insulin concentration, antioxidant activity (SOD and CAT) were analysed using SPSS version 25 using ANOVA followed by Post Hoc LSD comparison. Values were considered significant at $p<0.05$. The result showed significant ($p<0.05$) increase in the blood glucose level in groups B, C, D, E compared to group A at Day 0. At day 7, 14, 21 the result showed significant ($p<0.05$) decrease in the treated groups compared to diabetic control group. Insulin level had a significant ($p<0.05$) increase in treated groups compared to diabetic control group. There is significant increase in superoxide dismutase and catalase level in diabetic treated groups when compared to diabetic control group. The study concluded that the aqueous seed extract of *Aframomum melegueta* can be of immense use in phytomedicine especially for the management of diabetes mellitus.

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KEYWORDS: *Aframomum Melegueta, Diabetes Mellitus, Alloxan Monohydrate, Insulin Concentration, Antioxidants activity*

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterised by hyperglycemia and alteration in carbohydrate, fat and protein metabolism and it is associated with absolute or relative deficiencies in insulin secretion or insulin action (3). The prevalence of diabetes has reached an epidemic proportion worldwide. It is estimated that about 2.8% of the world population suffer from diabetes, according to world health organization. (19). The prevalence of Diabetes is increasing rapidly and it is anticipated that by the year 2030, it will double the current figure (16). This rapid increase in the prevalence of the disease is associated with rapidly changing lifestyle and environmental factors (11).The general characteristic of diabetes mellitus Include glucosuria, osmotic diuresis, polyuria, polydipsia and polyphagia.

Glucose is the primary source of energy for the body cells, it is transported from the intestine or liver to body cells via the bloodstream and is made available

for cells absorption via the hormone insulin, produced by the body primarily in the pancreas. Abnormalities in plasma lipids and lipoprotein patterns due to defect in insulin insufficiency has been well documented, in both type I and type II diabetes mellitus .Plasma glucose level is the main regulator of insulin secretion. However, a change in the concentration of plasma glucose that occurs in response to feeding or fasting is the main determinant of insulin secretion. In addition, destruction of the beta cells by diabetogenic substances will therefore cause a rise in blood glucose level.

The standard method of inducing diabetes in animal model is with Alloxan monohydrate, which is chemically known as 5, 5-dihydroxyl pyrimidine-2,4,6-trione (12) and is one of the common diabetogenic agents often used to assess the antidiabetic potential of both pure compounds and plant extracts in studies involving diabetes. The diabetogenicity of alloxan is underlined by its selective cellular uptake by beta cells of the pancreas and consequent accumulation in these cells (18). Alloxan monohydrate exhibits its diabetic actions in two distinct ways through either oxidative stress or inhibition of insulin synthesis, thus causing a sharp rise in blood glucose level (9). Several antidiabetic drugs are available to decrease blood glucose levels, even though their mechanism of action are variable. There is yet no effective cure for diabetes and the available drugs and insulin currently used in managing the diseases are associated with several undesirable side effects (6). The undesirable side effects and high cost of antidiabetic drugs has led to search for plants with hypoglycemic properties and their employment in management of diabetes (20).

An imbalance between reactive oxygen species (ROS) and antioxidants leads to oxidative stress resulting in excessive ROS generation, which induces lipid peroxidation. The antioxidant act as a defence mechanism that protect human against dangerous effects of oxidative reaction produced by reactive oxygen species (ROS) in a biological system Antioxidant defends the body against extreme reactive oxygen species (ROS) levels via enzymatic (superoxide dismutase, catalases, and peroxidases) and non-enzymatic mechanisms Overproduction of reactive oxygen species (ROS) or inadequate antioxidants has been implicated in the pathogenesis of some disease conditions like diabetes, Alzheimer's disease, cancer, atherosclerosis, arthritis, neurodegenerative disease, and aging process (17).

Herbal medicines involve the integration of several therapeutic experiences and practices of indigenous systems of medicine that may span many previous generations, which often provide valuable guidelines to the selection, preparation, and application of herbal formulation with a view to providing therapeutic benefits (7). Medicinal plants have been used since ancient times for the treatment and management of diabetes mellitus (DM) in traditional medicine systems of many cultures throughout the world. However, medicinal plants continue to play an important role in the management of diabetes mellitus, especially in developing countries, where many people do not have access to conventional anti-diabetic therapies (2). In developed countries, the use of anti-diabetic herbal remedies has been on the decline since the introduction of insulin and synthetic oral hypoglycemic drugs during the early part of the 20th century. Control of diabetes mellitus by species and other natural product is becoming popular and is more appropriate and economical for use in developing countries like Nigeria. Several species of medicinal plant used in traditional treatment and management of diabetes worldwide have been evaluated. Their hypoglycemic properties used in management of diabetes have been reported to be due to their contents of flavonoids, glycosides, alkaloids terpenoid and other bioactive compound.

*Aframomum Melegueta (*Grain of paradise) is a spice with similar composition as a ginger that belong to the same zingiberaceae family. *Aframomum Melegueta* is a perennial herbal plant that is cultivated because of its valuable medicinal and pharmacological effects such as antimicrobial, hepato-protective, anti-cancer and anti-diabetic effect **(8,14,15).**

Materials and Method

Phytochemical screening: The phytochemical screening was performed on the pulverized seeds of Aframomum melegueta for identification of phyto-constituents. The constituents tested include: Alkaloids, Tanins, saponins, phenolic compounds, flavonoids and steroids as described by panday (2014).

Location of the study: This study was carried out in the Animal House, Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus.

Ethical Approval: Ethical approval was obtained from the Faculty of Basic Medical Science, College of Health Science, Nnamdi Azikiwe University, Nnewi campus. Rats handling and treatments conform to guidelines of the National Institute of Health (NIH publication 85-23, (1985) for laboratory animal care and use.

Materials: *Aframomum melegueta (*Alligator pepper) Seed, Alloxan Monohydrate (Sigma Aldrich, USA),

Glucometer (Accu-check active, Mannheim, Germany), Glucose strips (Accu-check strips),S. Pyrex Beakers (Techmel, USA), Measuring cylinder (MINGHE), 5ml hypodermic syringe, Oral cannula, Filter paper (Whatman Qualitative Filter Paper No. 1, Sigma Aldrich WHA1001042), Electric blender, Magnetic stirrer, Distilled water, Standard Plastic Cages, water can, Cotton wool (KENS LINT, Benin City, Nigeria), Latex Medical Hand gloves (Supermax Gloves, Selangor, Malaysia), Diethyl ether, Vital top feed grower (JOS, Nigeria), Plain Container Organ Bottles, Microhematocrit Centrifuge (SH120), Nexus Refrigerator, Rotary evaporator (Digital) TT-52 (Techmel & Techmel, USA), Thermostat Oven (DHG-9023A, PEC MEDICAL USA), Heparinized Capillary Tube, Radioimmunoassy kit.

Chemicals: Alloxan monohydrate (Sigma Aldrich, USA)

Plant Collection: The seeds of *Aframomum Melegueta* was obtained from local market in Onitsha. The Department of Botany, Nnamdi Azikiwe University, Awka, Anambra State identify botanical identification of the seed and the herbarium number (FHI108876) was deposited in the herbarium catalog.

Preparation of Seed Extract: Seeds of *Aframomum melegueta* was washed with water and air-dried under the shade to constant weight. The seeds was ground into powdery form with an electric blender. The 20g of the powdered seed was soaked in 100ml of distilled water with constant stirring by a magnetic stirrer for 48 hours. It was filtered using a muslin cloth and futher filtration using what man No1 filter paper. The filtrate was concentrated using a rotatory evaporator and was stored in a refrigerator at 8oC, where stock solution was prepared.

Experimental Animals: Twenty five male wistar rats weighing 150- 170g was obtain from the the Animal House, Department of Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus. Animals were kept in standard cages at a room temperature of 27±2°C. The animals were kept on 12hours light and dark cycle and were maintained with normal laboratory chow (Grower feed) and water *ad libitium*. The animals were acclimatized for two weeks before the administration of aqueous seed extract of *Aframomum Melegueta.*

Experimental Design: Twenty-five (25) male Wistar rats weighing 150-170g was used for this study. The animals were acclimatized for a period of two weeks, after which the animal were randomly divided into 5 groups of 5 animals each.

Group A - served as Negative Control (Animals received only distilled water and laboratory chow).
Group B - served as positive control (Animals received only Alloxan monohydrate).
Group C - received 120mg/kg of Alloxan Monohydrate and treated with 150mg/kg of aqueous seed extract of *Aframomum Melegueta.*
Group D - received 120mg/kg of Alloxan Monohydrate and treated with 300mg/kg of aqueous seed extract of *Aframomum Melegueta.*
Group E - received 120mg/kg of Alloxan Monohydrate and treated with 600mg/kg of aqueous seed extract of *Aframomum Melegueta.*
The Administration of the extract last for a period of 21 days and the administration was between the period of 6 am to 8 am every day.

Induction of Diabetes Mellitus: Diabetes Mellitus (Hyperglycemia) was induced in the experimental rats by injecting 120 mg/kg of Alloxan monohydrate intra-peritoneally dissolved in 0.9% cold normal saline to overnight-fasted rats (12 h). The rats were kept for the next 24hours on 10% glucose solution bottles, in their cages, to prevent hypoglycemia. After 72hours of injection, fasting blood glucose level was measured using glucometer (Accu Check). Random blood glucose ≥ 200mg/dl indicates diabetes mellitus (10). The animals that did not develop more than ≥ 200mg/dl glucose levels was removed from the study.

Blood Glucose Estimation: Fasting blood glucose was determined using glucometer kit (accu-check) after over-night fasting for 12hours. The tail of the wistar rats were punctured, using lancet and blood from the tail were dropped on the strips, which was inserted into the glucometer and blood glucose concentration in mg/dl for each rat in various groups were recorded.

Sample Collection: At the end of the experiment, animals in the different groups were anesthetized using chloroform in an enclosed container after 24-hours of the last administered dose of the aqueous seed extract of *Aframomum melegueta*. Blood were collected from the animals using a heparinized capillary tube through ocular puncture as described by Parasuraman, Raveendran, and Kesavan (2010). Blood obtained was put in a plain bottle, allowed to cool, and centrifuged for 10-minutes at 3000rpm, after which the serum was retrieved using a micropipette. The retrieved serum was used to assay for Insulin Concentration and Antioxidant activity.

Determination of Insulin Concentration: Serum insulin concentration was determined using radioimmunoassay kits according to the method of Marschner *et al*.

Estimation of Superoxide Dismutase Activities: The serum obtained was used to assay for Superoxide Dismutase (SOD) activity by the method described by Zhang *et al.*(21)

Statistical Analysis: Data obtained was analyzed using One Way Analysis of Variance (ANOVA), followed with post Hoc LSD to determine the level of significance between the control and experimental groups. The values were expressed as Mean ± Standard Error of Mean (Mean±SEM) and the difference was considered statistically significant at $p<0.05$. All statistical analysis was performed using statistical package for social sciences (SPSS) version 25.

RESULTS AND DISCUSSION

Table 1: Effect of aqueous seed extract of *Aframomum melegueta* on blood glucose level at day 0, 7, 14, and 21 following alloxan toxicity

	Blood glucose day 0 (mg/dl)	Blood glucose day 7 (mg/dl)
	MEAN±SEM	MEAN±SEM
Group A (control)	79.67±0.88	78.33±1.76
Group B (Diabetic control)	430.33±46.94*	411.67±33.45*
Group C (Dm +150mg/kg of ASAM)	359.00±37.26*	191.67±7.26*
Group D (DM+300mg/kg of ASAM)	382.67±36.49*	186.67±12.01*
Group E (Dm+ 600mg/kg of ASAM)	358.00±35.23*	186.66±14.52*
F-value	15.56	48.52

Data was analyzed using ANOVA followed by post Hoc multiple Fisher's LSD comparisons and values were considered significant at $p<0.05$. SEM: Standard error of mean, DM: Diabetes mellitus, ASAM: aqueous seed extract of *Aframomum melegueta*, significant (*) and not significant (^{NS}).

Table 1 result revealed a significant increase in the blood glucose level in groups B, C, D, and E ($p=0.00, p=0.01, p=0.03, p=0.01$) compared to A at Day 0. At Day 7, a significant decrease was indicated in groups C, D, and E compared to B ($p=0.02, p=0.00, p=0.04$), while group B had a significant ($p=0.01$) increase in the blood glucose level compared to A.

Table 2: Effect of aqueous seed extract of *Aframomum melegueta* on blood glucose level at day 14 and 21 following alloxan toxicity

	Blood glucose day 14 (mg/dl)	Blood glucose day 21 (mg/dl)
	MEAN±SEM	MEAN±SEM
Group A (control)	78.67±2.02	77.33±1.45
Group B (Diabetic control)	420.00±41.63*	393.33±51.75*
Group C (Dm +150mg/kg of ASAM)	118.33±4.40*	91.67±6.67*
Group D (DM+300mg/kg of ASAM)	113.33±6.11*	93.67±2.84*
Group E (Dm+ 600mg/kg of ASAM)	101.33±3.18*	92.33±4.33*
F-value	**57.16**	**33.80**

Data was analyzed using ANOVA followed by post Hoc multiple Fisher's LSD comparisons and values were considered significant at $p<0.05$. SEM: Standard error of mean, DM: Diabetes mellitus, ASAM: aqueous seed extract of *Aframomum melegueta*, significant (*) and not significant (^{NS}).

At Day 14, a significant decrease was indicated in groups C, D, and E compared to B ($p=0.01, p=0.01, p=0.03$), while group B had a significant ($p=0.00$) increase in the blood glucose level compared to A. At Day 21, a significant decrease was indicated in groups C, D, and E compared to B ($p=0.01, p=0.03, p=0.02$), while group B had a significant ($p=0.02$) increase in the blood glucose level compared to A

Table 3: Effect of aqueous seed extract of *Aframomum melegueta* on insulin level following alloxan toxicity

	Insulin level (uiU/ml)
	MEAN±SEM
Group A (control)	39.08±0.85
Group B (Diabetic control)	28.51±6.95^{NS}
Group C (Dm +150mg/kg of ASAM)	62.39±5.04*
Group D (DM+300mg/kg of ASAM)	49.32±3.60*
Group E (Dm+ 600mg/kg of ASAM)	48.41±5.19*
F-value	**6.96**

Data was analyzed using ANOVA followed by post Hoc multiple Fisher's LSD comparisons and values were considered significant at $p<0.05$. SEM: Standard error of mean, DM: Diabetes mellitus, ASAM: aqueous seed extract of *Aframomum melegueta*, significant (*) and not significant (^{NS}).

Table 3 result showed an insignificant decrease in insulin level in-group B compared to A ($p=0.15$); in contrast groups C, D, and E had a significant ($p=0.00, p=0.01, p=0.02$) increase in insulin levels compared to B.

Table 4: Effect of aqueous seed extract of *Aframomum melegueta* on superoxide dismutase and catalase level following alloxan toxicity

	Superoxide Dismutase (U/ml)	Catalase level (IU/L)
	MEAN±SEM	MEAN±SEM
Group A (control)	9.41±0.05	43.56±0.68
Group B (Diabetic control)	4.75±0.72*	27.70±1.18*
Group C (Dm +150mg/kg of ASAM)	10.61±0.36*	61.45±1.62*
Group D (DM+300mg/kg of ASAM)	10.56±1.34*	52.02±1.71*
Group E (Dm+ 600mg/kg of ASAM)	10.32±1.27*	64.75±0.45*
F-value	**7.61**	**76.45**

Data was analyzed using ANOVA followed by post Hoc multiple Fisher's LSD comparisons and values were considered significant at $p<0.05$. SEM: Standard error of mean, DM: Diabetes mellitus, ASAM: aqueous seed extract of *Aframomum melegueta*, significant (*) and not significant (^{NS}).

Table 4.5 result showed a significant decrease in superoxide dismutase level in-group B compared to A ($p=0.01$), while groups C, D, and E had a significant increase in superoxide dismutase level ($p=0.01, p=0.01, p=0.01$) compared to B. The catalase level showed a significant decrease in group B compared to A ($p=0.02$), while groups C, D, and E had a significant increase in Catalase level ($p=0.00, p=0.01, p=0.03$) compared to B.

Presentation of Results

Phytochemical Screening results of aqueous seed extract of *Afromomum melegueta*

Test	Sample (aqueous seed extract of *Afromum melegueta*)
Alkaloids	+++
Flavonoids	+++
Saponins	+++
Phenols	++
Tannins	+
Terpenoids	++
Steroids	-

+++ (Highly present), ++ (moderately present), + (mildly present), and – (absent)

Result revealed the presence of alkaloids, saponins, flavonoids, terpenoids, phenols and tannins while steroids were absent.

DISCUSSION

The occurrence of diabetes mellitus has become a serious threat to the health of mankind globally, hence the urge to develop solutions with little or no side effects and at a cheaper rate. Natural products in form of plant preparations have been used in the treatment and control of wide array of diseases. This study has evaluated the effects of aqueous seed extract of *Aframomum Melegueta* on blood glucose, Insulin concentration in alloxan induced diabetes in male wistar rat.

The result of the phytochemical Screening of the dried seeds sample of *Aframomum melegueta* as carried in this study revealed the presence of alkaloids, flavonoids saponins, tanins, phenols and terpenoids in various quantities while steroid was absent. These phytochemical agents have indeed been implicated in the treatment of various diseases (4)It has been reported that flavonoids, tannins, and saponins possess hypoglycaemic properties through an inhibitory action on the sodium-glucose transporter 1 (S-GLUT1) (26).The presence of these bioactive compounds was earlier reported by previous studies that flavonoids have anti-hyperglycemic properties because they stimulate glucose uptake in peripheral tissues and attenuate oxidative stress during diabetic conditions (3)

In the diabetic group (B-E), the increase in blood glucose level are indicative of hyperglycemic effect of alloxan resulting from it ability to damage the insulin secreting cells of the pancreas.These cells are sensitive to cytotoxic action of which induces

experimental diabetes in animals (1). It accumulates at the cytosol of the beta-cells as glucose analogues thereby destroying the cells thus leading to hyperglycemia (13). This coincides with the work done by Mahesar *et.al* (18) and Morakinyo*et.al* (20), that administration of alloxan(120mg/kg) led to 3fold elevation of fasting blood glucose level which was maintained over a period of two weeks.

The study further revealed that on Day 7 treatment with *Aframomum melegueta* seed extract (150, 300 and 600 mg/kg), the Blood Glucose level in all the diabetes induced groups got reduced. This suggests that the aqueous seed extract of *Aframomum melegueta* has hypoglycaemic activity. Also on day 14 and 21 the result showed that the blood glucose level in the diabetic rats treated with aqueous seed extracts of *Aframomum Melegueta* significantly decreases as the study duration increases. Especially at highest dose of 600mg/kg, this is due to high concentration of the active Phytochemical in the extract. This result agrees with the work done by llic *et al*. (15) Olaitan *et.al* (22) and Adetoun *et.al* (3) that flavonoids have anti-hyperglycemic properties because they stimulate glucose uptake in peripheral tissues and attenuate oxidative stress during diabetic conditions.

The untreated diabetic group(B) with highest blood glucose level show insignificant decrease of insulin level when compared to control and other diabetic group treated with aqueous seed extract of *Aframomum melegueta*. This decrease in insulin level in untreated diabetic group is because alloxan causes diabetes in rats by damaging the insulin-secreting cells of the pancreas.

On treatment with aqueous seed extract of *Aframomum melegueta ,* the Insulin level increased significantly when compared to the untreated diabetic group. But among the *Aframomum Melegueta* treatment groups, Group E showed highest insulin level.This agree with the work done by Adetoun *et.al.,* (3) that Flavanoids have been actively involved in the restoration of pancreatic β-cell and insulin secretion.Saponins are known to be efficiently involved in the restoration of pancreatic β-cell and insulin secretion (11). Aminu *et.al.,* (7) demonstrated a similar report to the study findings that improved serum insulin levels in the*Aframomum Melegueta* treated diabetic animals could be due to the regeneration of pancreatic islets observed in the pancreatic histology.

CONCLUSION

In conclusion, the aqueous seed extract of *Aframomum melegueta* can be of immense use in phytomedicine especially for the management of diabetes mellitus. The aqueous seed extract of *Aframomum Melegueta* showed hypoglycemic effects and also increase insulin levels.

Hence, this research study shows that the aqueous seed extract of *Aframomum Meleguetae* is safer and also had ameliorative effect in insulin concentration seen in diabetes mellitus.

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